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Liquid chromatography–electrospray ionization isotope dilution mass spectrometry analysis of paraquat and diquat using conventional and multilayer solid-phase extraction cartridges

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Abstract

The performance of alkyl-silica sorbent packed solid-phase extraction (SPE) cartridges and a mixed-mode, polymeric sorbent packed SPE cartridge (resin SPE cartridge) were evaluated for the sample preparation of paraquat and diquat in environmental water and vegetation matrices. Also the recoveries of the native and ^{2}H-labeled paraquat and diquat were correlated to validate that the ^{2}H-labeled species can be used for the isotopic dilution mass spectrometry (IDMS) analysis of paraquat and diquat. The results show that the extraction efficiency of alkyl-silica SPE is dependent on the carbon loading of the sorbent and deteriorates with an increasing sample pH. The resin SPE cartridge required no pH adjustment and showed excellent correlation between the native and ^{2}H-labeled species, therefore, allowing us to develop the first liquid chromatography–electrospray ionization IDMS analytical method for the analysis of paraquat and diquat in environmental water and vegetation matrices. Method detection limits derived using standard EPA protocol were 0.2 and 0.1 μg/l for paraquat and diquat in water matrices, and 0.02 and 0.01 μg/g in vegetation matrices, respectively.

Keywords: Mass spectrometry; Solid-phase extraction; Isotope dilution mass spectrometry; Pesticides; Paraquat; Diquat

1. Introduction

1,1′-Dimethyl,4,4-bipyridinium (paraquat) and 1,1′-ethylene-2,2′-bipyridinium (diquat) are fast-acting, quaternary ammonium, non-selective, contact herbicides which inhibit photosynthesis when applied to plant foliage. They are used extensively as pre-harvest desiccants and defoliants, and also for industrial and aquatic weed control. Although they are highly water soluble, they will not easily be leached from soil or taken up into plant root systems as they are quickly and strongly adsorbed to clay and soil organic matters. Various methods have been used for the analysis of paraquat and diquat in vegetation and water samples. These include gas chromatography (GC) [1–3], immunochemical assay [4,5], liquid chromatography (LC) with UV detection [6–14] or mass spectrometry (MS) [15–21], and more recently, electrophoretic methods with UV detection [22–30].

Historically, ion-pairing (IP) extraction using an alkyl-silica solid-phase extraction (SPE) cartridge or disk, followed by LC–UV analysis has been used for the determination of paraquat and diquat in water, crop, and vegetation samples [6–14]. The lack of specificity of the UV detection method prohibits the

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use of isotopically labeled method surrogates to closely monitor and compensate for any errors during the analysis [31]. Of late, the availability of reliable liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS) has lead to the development of methods using ESI as an alternative to UV detection for the analysis of paraquat and diquat [15–21]. ESI-MS based methods offer superior sensitivity, mass specific data, and allows the use of isotopically labeled surrogates. Two of these recently developed methods [17,18] use ^{2}H-labeled paraquat and diquat as internal standards to improve data quality, however, none was validated for isotope dilution mass spectrometry (IDMS) quantitative analysis. Also, these new LC–ESI-MS methods [15–21] continue to use IP based alkyl-silica SPE cartridge sample preparation procedures and are susceptible to the inconsistent quality of alkyl-silica sorbent because of the batch-to-batch variation of silica materials [32]. The pH modification process, which is an integral part of alkyl-silica sorbent based SPE methods for paraquat and diquat SPE extraction, can also affect method efficiency since analyte retention will be weak if the sample pH is not optimized [33].

The mixed-mode polymeric SPE (resin SPE) cartridges, depending on the functional groups bonded to its polymeric backbone, can have both reversed-phase and ion-exchange characteristics. Details on the type and application of resin cartridges are available from Waters (www.waters.com) and International Sorbent Technology (www.ist-spe.com). The mixed mode functionality eliminates the need for IP-based alkyl-silica SPE and provides an opportunity to develop a simple and rugged alternative for paraquat and diquat analysis. Combining this new extraction method, ^{2}H-labeled method surrogates, and LC–ESI-MS instrumentation, we have developed the first LC–ESI-IDMS method for the quantitative determination of paraquat and diquat with confidence.

In this study the alkyl-silica and resin SPE cartridges were evaluated for use with the IDMS analysis of paraquat and diquat. We evaluated the effect of pH on the recovery and correlation between the native and ^{2}H-labeled paraquat and diquat for the alkyl-silica SPE cartridges. In addition, we critically evaluated the behavior of $^{2}H_{8}$-paraquat and $^{2}H_{4}$-diquat in environmental matrices and the feasibility for their use as internal standards for LC–ESI-IDMS paraquat and diquat analysis using the resin SPE cartridge. Quality assurance data compiled from 6-month of quality control samples was used to validate the method.

2. Experimental

Due to the high ionic strengths of paraquat and diquat, all glassware used in this work were silanized using Supelco Sylon CT silanizing solution (Supelco, Mississauga, Canada) to prevent the analytes from adsorbing to the glassware walls. Whenever possible, polypropylene labware was used to alleviate this concern. Water and vegetation samples were collected routinely from across the Province of Ontario in 500-ml plastic bottles and stored at 4 ± 2 °C prior to sample preparation. Laboratory tap water and corn leaves were used for water and vegetation quality control samples.

2.1. Chemicals and reagents

Diquat dibromide monohydrate (99%) and paraquat dichloride tetrahydrate (99%) were obtained from Sigma–Aldrich (Mississauga, Canada). $^{2}H_{8}$-paraquat and $^{2}H_{4}$-diquat (both 98% isotope enriched) were obtained from CDN Isotopes (Montreal, Canada). Certified reference standard solutions of diquat and paraquat were obtained from ChemService (West Chester, PA, USA), Sigma–Aldrich, and Dr. Ehrenstorter (Augsburg, Germany). Ammonium chloride (99.5%) and ammonium hydroxide, ACS, 30% in water, were obtained from Anachemia Canada (Montreal, Canada); trifluoroacetic acid, (99%) was obtained from Aldrich (Milwaukee, WI, USA). Cetyltrimethylammonium bromide, 95%, and 1-hexanesulfonic acid, sodium salt, 98%, were obtained from Supelco (Oakville, Canada). All solvents were HPLC or distilled in glass grade and were obtained from Caledon Labs. (Georgetown, Canada). Oasis MCX 6 ml, 150 mg resin SPE cartridges were obtained from Waters (MA, USA). Alkyl-silica SPE cartridges with C_{8}- and C_{18}-alkyl chains were obtained from Supelco Canada (Oakville, Canada), Worldwide Monitoring (Mississauga, Canada), and

Table 1
Characteristics of the alkyl-silica SPE cartridges evaluated and typical recovery ($n \geq 3$) of paraquat and diquat

SPE cartridge	Alkyl chain type	Carbon loading (w/w, %)	Method recovery			
			Paraquat		Diquat	
			Average (%)	RSD (%)	Average (%)	RSD (%)
1	C_8	7	84	2.6	80	3.5
2	C_8	11	73	1.3	75	4.4
3	C_8	14	41	11	43	12
4	C_{18}	11	92	2.3	87	1.2
5	C_{18}	17	40	2.6	31	0.7
6	C_{18}	21	44	16	32	9.1

Chromatographic Specialties (Brockville, Canada). Table 1 lists the characteristics of these alkyl-silica SPE cartridges.

For the resin SPE cartridge, 1 *M* ammonium chloride solution in methanol–deionized water (50:50) was used as the cartridge elution solution. Alkyl-silica SPE cartridge conditioning solutions were prepared according to the EPA Method 549 [6]. Cartridge elution solution for the alkyl-silica SPE cartridge was 1.35% TFA in water. The LC mobile phase for all analyses was 25 m*M* TFA in methanol–deionized water (5:95), prepared by diluting 1.9±0.05 ml TFA and 50 ml methanol in 948 ml of deionized water.

2.2. Calibration solutions

Individual stock standards of paraquat, diquat, $^2H_8$-paraquat, and $^2H_4$-diquat were prepared in deionized water at a cation concentration of 1000 μg/ml. Before weighing, the salts were dried for 4 h at approx. 110 °C and cooled to ambient temperature in a desiccator. Intermediate standards, 100 μg/ml in deionized water, were prepared from the stock solutions. Calibration standard mixtures were prepared from the intermediate standards, covering the range of 0.01–1.0 μg/ml for paraquat and 0.005–0.5 μg/ml for diquat. The internal standards, $^2H_8$-paraquat and $^2H_4$-diquat, were maintained at concentrations of 0.15 and 0.10 μg/ml, respectively. The calibration standards were validated and controlled at 100±5% against three certified reference standard solutions, prior to use.

2.3. Sample preparation

2.3.1. Water samples

This procedure was validated using pure water, chlorine treated drinking water, ground water, surface water, and well water. Resin SPE cartridges were conditioned using 5 ml of water, followed by 5 ml of methanol, and another 5 ml of water. Prior to extraction, a known amount of method surrogates ($^2H_8$-paraquat and $^2H_4$-diquat) was added to 200 ml of water sample and mixed well. Samples were extracted by vacuum aspiration through the SPE cartridges at a flow-rate of approx. 3 ml/min. After extraction, the cartridges were rinsed with 5 ml methanol–deionized water (50:50), and air dried for approx. 1 min. Analytes were then eluted from the SPE cartridges using 5 ml of cartridge elution solution in two steps. The resin was first wetted with 1 ml of elution solution for approx. 1 min followed by 4 ml of elution solution to complete the elution process. An aliquot of the extracts were filtered through 0.45 μm nylon filters into a 1.0-ml polyethylene sample vial and analyzed by LC–ESI-MS. We found that with the resin SPE cartridges, sample extraction and elution required careful flow-rate control. Flow-rates that were too high, i.e., greater than 5 ml/min, resulted in sample breakthrough or incomplete retention of the analytes.

For the alkyl-silica SPE cartridges, samples were prepared according to the EPA method 549 [6] with the addition of ^2H-labeled paraquat and diquat as method surrogates. To maximize the sensitivity of the ESI-MS analysis, the eluent used was 1.35% TFA aqueous solution.

2.3.2. Vegetation samples

Vegetation samples for paraquat and diquat analysis were flash frozen using about 10 ml of liquid nitrogen. The frozen sample was blended at maximum speed in a Waring blender for approx. 60 s. Approx. 2 g of the homogenized sample was accurately weighed, fortified with the method surrogates, and extracted for 2 min in the blender with 40 ml of 0.5 *M* HCl and 20 ml of dichloromethane. The resulting mixture was centrifuged in a glass centrifuge tube at 3500 rpm for 20 min. The aqueous supernatant was transferred to a 500-ml plastic bottle, diluted to 200 ml with deionized water, the pH was adjusted to 6.5±0.5 using 10% NaOH, and analytes were extracted using the resin SPE cartridges.

2.4. Instrumentation and analytical procedures

A HP 1100 Modular LC–MS system (Agilent Technology, Palo Alto, CA, USA) was used for sample analysis. Chromatographic separation was achieved at 15 °C on a Zorbax SB-C3, 150×4.6 mm, 5.0 μm, non-endcapped, analytical column. The isocratic LC separation was carried out at a flow-rate of 0.75 ml/min. The injection volume for the analysis was 20 μl and the total analysis time was 6 min.

The ESI interface used N_2 gas at a pressure of 40 p.s.i. and flow-rate of 12 l/min (1 p.s.i.=6894.76 Pa). The drying gas temperature and capillary voltage were set at 350 °C and 3500 V. The MS system was tuned daily using an Agilent proprietary tuning mixture. MS data were acquired in the positive ionization mode using selected ion monitoring and a fragmentation voltage of 90 V. Identification of paraquat and diquat was achieved using LC retention times calculated from the selected ion monitoring plots (SIMPs), one target ion, one qualifier ion, and the ratio of area counts of the target and qualifier ions. Integrated areas obtained from the SIMPs of the target ions were used for quantification using IDMS protocol [31].

The progress of the LC analysis was monitored using the diode-array detector (DAD) at wavelengths 308 nm for diquat/$^2H_4$-diquat and 257 nm for paraquat/$^2H_8$-paraquat. The DAD data was used to monitor the presence of excess contamination in a given sample and, as a secondary confirmation tool for paraquat and diquat analysis. By constantly evaluating the DAD data it was possible to keep sample-to-sample carry over and ESI-MS contamination to a minimum, thus, reducing MS downtime. The linear range of the LC–ESI-MS system for paraquat and diquat was demonstrated to be at least three orders of magnitude, corresponding to 0.1 to 100 ng on-column. Typical loadings of paraquat and diquat in the real world samples ranged from <0.1 ng (drinking water samples) to greater than 200 ng on-column (vegetation samples). Routine analysis of paraquat and diquat was calibrated using a three-point calibration curve with a column loading of 0.2, 5, and 10 ng.

3. Results and discussion

Mass spectra of native and ^2H-labeled paraquat and diquat are shown in Fig. 1. During the LC–ESI-MS analysis, *m/z* 185 and 93 were monitored for paraquat, *m/z* 183 and 92 were monitored for diquat, and *m/z* 193 and 186 were monitored for $^2H_8$-paraquat and $^2H_4$-diquat, respectively. Typical reconstructed LC SIMP chromatograms are shown in Fig. 2. Current LC parameters allows a baseline separation of paraquat and diquat within 6 min. This baseline separation is also required for the unambiguous identification and quantitation of $^2H_4$-diquat as, from Fig. 1, native paraquat has a significant fragment at *m/z* 186 and could affect the area counts of $^2H_4$-diquat if not baseline separated.

3.1. Alkyl-silica SPE cartridges—effect of carbon loading and sample pH

The extraction efficiency of the IP based alkyl-silica SPE cartridge can be affected by factors such as the sample pH and the level of carbon loadings on the alkyl-silica sorbent. Using laboratory control samples and six different alkyl-silica SPE cartridges, we first investigated the effect of carbon loading on method performance for paraquat and diquat analysis. As shown in Table 1, the recovery of paraquat and diquat was found to be inversely proportional to the degree of carbon loading of the alkyl-silica sorbent. This result can be explained using the ion-

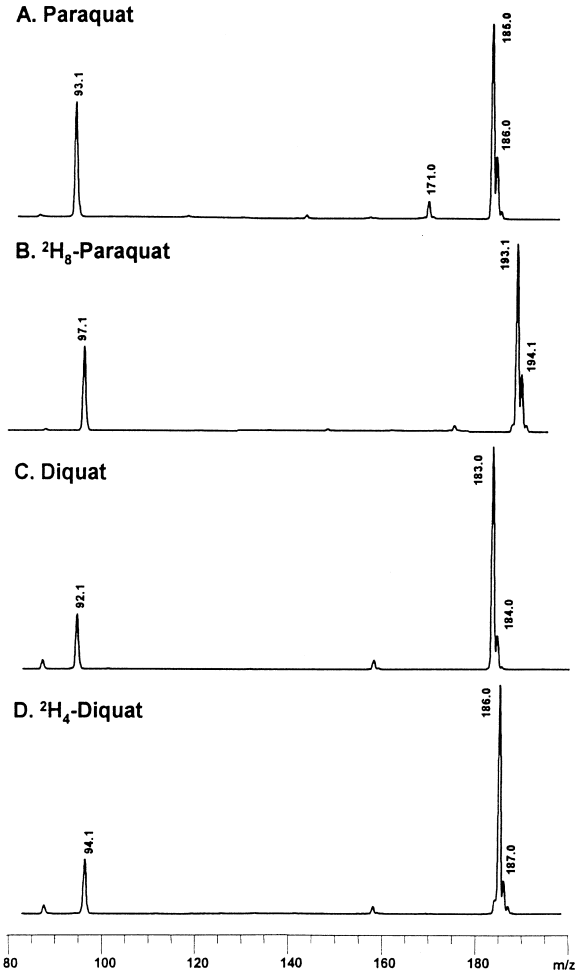

Fig. 1. Reconstructed LC SIMP plots obtained from MS spectral data. Native paraquat (dotted line) and diquat (solid line), $^2H_8$-paraquat (thin dashed line, A_{peak}=2.876 min), and $^2H_4$-diquat (dashed line, A_{peak}=3.204 min).

interaction model of paired ion system proposed by Bidlingmeyer et al. [34] and Warren [35]. Paraquat and diquat are extracted via the lipophilic interaction of their aromatic moiety and the C_8- and/or C_{18}-chains and/or the electrostatic attraction between the IP reagent and paraquat and diquat ions. The electrostatic attraction is the dominant force and increases in proportion with the decrease of carbon loading. Therefore, the extent to which paraquat and diquat are extracted from the sample is inversely proportional to the carbon loading, regardless of the alkyl chain length of the SPE sorbent. As shown in Table 1, cartridges 1 and 4 have different alkyl chain lengths and the lowest carbon loading in their peer groups (C_8- and C_{18}-alkyl chains), and give the best performance for the recovery of the analytes.

Using alkyl-silica SPE cartridges 1 and 4, we investigated the effect of pH on the recovery of native paraquat and diquat and $^2H_8$-paraquat and $^2H_4$-diquat, specifically at pH 5.0, 7.0, 8.5, and 10.0. This was done by extracting and analyzing a set of fortified water samples immediately after pH adjustment. We found that an increase in pH values adversely affected the recovery of both the native and deuterated analytes, see Fig. 3. From the figure, it is clear that while the recoveries of native paraquat and $^2H_8$-paraquat correlate well at all pH values, the recovered amount decreased from a high of over 80% at pH 5 to a low of approx. 40% at pH 10.0. Clearly, an increase in pH values has an identical and adverse effect on the native and $^2H_4$-diquat. Recoveries and correlation of native diquat and $^2H_4$-diquat are good at pH 5 and 7, but the recovery and the correlation deteriorated rapidly with the increasing pH. At pH 10.0, there is no recovery of the $^2H_4$-diquat and only 20% recovery of the native diquat. These results indicate that the efficiency of alkyl-silica SPE methods for paraquat and diquat is pH dependent.

Although the IDMS analysis of paraquat and diquat can be carried out at pH 5.0 and 7.0, we noticed that pH modification tended to cause precipitate formation in raw water, ground water and river water samples. These precipitates can eventually block the extraction cartridge making it very difficult to continue the extraction process. This study results show that when using alkyl-silica SPE cartridges, one must control sample pH and select alkyl-silica cartridges carefully to ensure a successful IDMS analysis.

3.2. IDMS analysis using resin SPE cartridges and ^2H-labeled paraquat and diquat

The complexities involved with alkyl-silica SPE cartridges led us to examine the use of a resin SPE cartridge for paraquat and diquat analysis. The goal was to achieve an efficient SPE sample preparation method that is amenable for use with ESI-MS

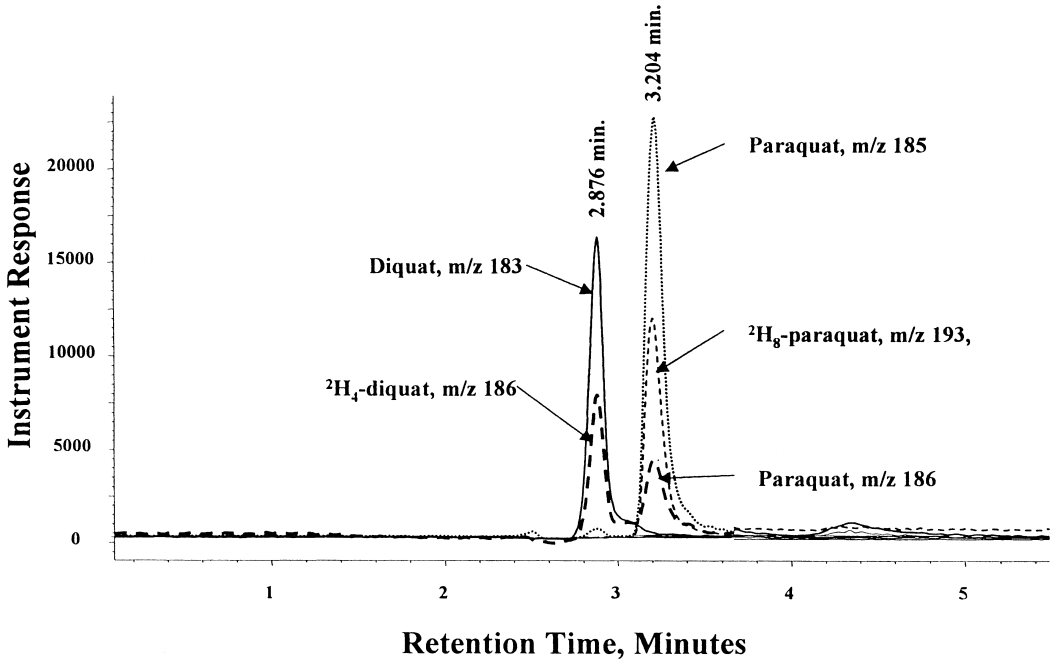

Fig. 2. ESI-MS spectra of paraquat (A), $^2H_8$-paraquat (B), diquat (C), and $^2H_4$-diquat (D).

analysis. Such a method should also allow the use of $^2H_8$-paraquat and $^2H_4$-diquat as method surrogates for ESI-IDMS analysis of paraquat and diquat, and not be susceptible to sample matrix effects. The suitability of $^2H_8$-paraquat and $^2H_4$-diquat for use as surrogate analytes in the IDMS analysis was first evaluated using fortified pure water laboratory control samples.

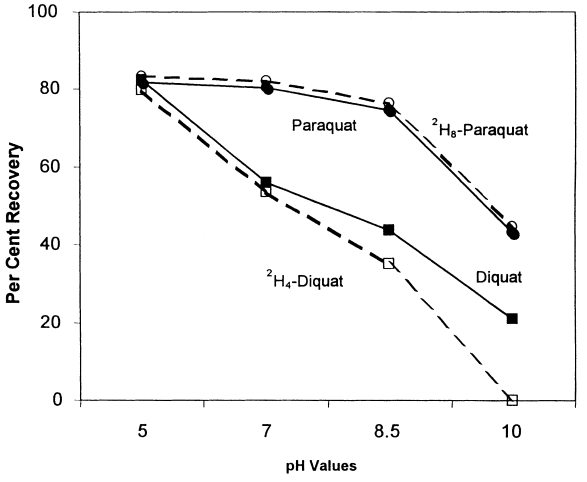

Fig. 3. Effect of pH values used during the sample extraction step.

IDMS analysis requires that the recovery of the target compound is equal to that of the surrogate. Therefore, when the recovery of the surrogates is plotted against that of the target analytes, the slope of the least-square fitted line should be 45°. Fig. 4 shows the correlation plots for the recoveries of paraquat and $^2H_8$-paraquat, and diquat and $^2H_4$-diquat for the laboratory control water spikes. These plots show a high level of correlation between the deuterated surrogates and target analytes, with a slope of 43.4° for paraquat and 50.2° for diquat. Compared to the external standard method of analysis, IDMS analysis improved the recoveries of paraquat and diquat from 80±10% and 82±13%, to 103±3% and 98±4%, respectively. The data clearly supports the use of $^2H_8$-paraquat and $^2H_4$-diquat as surrogate analytes for IDMS paraquat and diquat analysis.

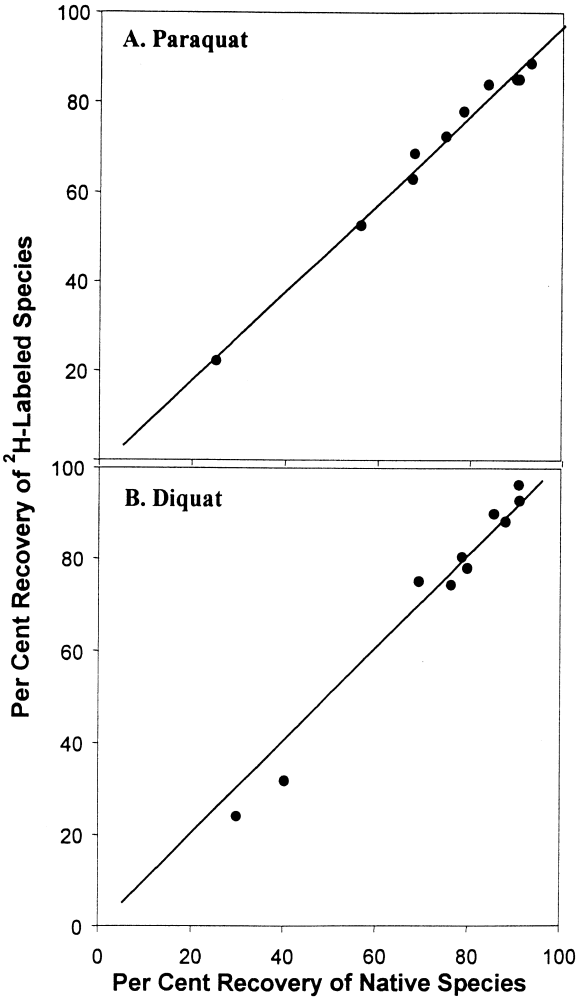

Fig. 4. Correlation plots for recoveries of paraquat and $^2H_8$-paraquat (A), and diquat and $^2H_4$-diquat (B), obtained from deionized water samples.

To further substantiate these results, we compiled recoveries of fortified composite raw water, chlorinated drinking water (tap water), and vegetation samples analyzed over a period of 6 months using the IDMS protocol. Table 2 shows the results of the IDMS evaluation for raw water, chlorinated drinking water, and vegetation samples. From Fig. 4 and Table 2, it is clear that excellent quality control data is obtained using ^{2}H-labeled paraquat and diquat as method surrogates for the preparation of environmental water and vegetation matrices using resin SPE cartridges and LC–ESI-IDMS analysis. Using the standard EPA protocol [36] the method detection limits (MDLs) for water were calculated to be 0.2 and 0.1 μg/l for paraquat and diquat, respectively. And for vegetation matrices they were calculated to be 0.02 and 0.01 μg/g for paraquat and diquat, respectively. These results are shown in Table 3.

It is worth mentioning that we observed different trends in the precision and accuracy data for vegetation sample matrices. Using $^2H_4$-diquat we achieved excellent correlation and precision data for vegetation IDMS analysis; however, the recoveries tended to be slightly high (from 82±22 to 119±7%). Without the availability of concrete evidence, a possible cause for this anomaly may be the enhanced deuterium/hydrogen exchange in the low pH environment of the extraction medium and/or LC mobile phase. This exchange would reduce the actual recovery of $^2H_4$-diquat, and therefore increase the recovery of native diquat as calculated using IDMS protocol. Further investigation, including the use of a neutral LC mobile phase and LC–MS–MS analysis of sample preparation by-products are under way to improve this method further.

Table 2
Summary of method performance obtained from quality assurance data

Sample description	*n*	Paraquat			Diquat		
		Recovery (%)		Slope of correlation line, degree	Recovery (%)		Slope of correlation line, degree
		Without IDMS quantitation	Via IDMS quantitation		Without IDMS quantitation	Via IDMS quantitation	
Raw water	13	59±23	102±10	45.5	68±11	96±9	46.6
Tap water	23	57±20	98±5	44.7	64±7	98±5	45.8
Vegetation	18	47±16	99±8	44.9	82±22	119±7	40.1

Table 3
Method recoveries and detection limits for paraquat and diquat in pure water and vegetation (lettuce) matrices

	Paraquat		Diquat	
	Water	Vegetation	Water	Vegetation
Spiking level (μg/l or μg/g)	2.5	0.1	1.25	0.1
n	8	9	8	9
Average recovery (μg/l or μg/g)	2.44	0.09	1.11	0.11
Recovery (%)	98	90	89	112
SD (μg/l or μg/g)	0.07	0.006	0.04	0.003
RSD (%)	3	7	4	3
MDL (μg/l or μg/g)	0.2	0.02	0.1	0.01

A 2.5-μg amount of paraquat and 1.25 μg of diquat were used to fortify the water matrix. A 5-μg amount of paraquat and diquat were used to fortify the vegetation matrix (5 g).

4. Conclusion

It is demonstrated that resin SPE cartridges can be successfully used to prepare samples for the LC–ESI-IDMS analysis of paraquat and diquat in aqueous and vegetation matrices. The resin SPE sample preparation method requires neither pretreatment for aqueous samples nor the use of ion-pairing agents. Method performance data obtained from the laboratory quality control and quality assurance samples showed outstanding precision and accuracy, although diquat results in vegetation matrices results were biased high by up to 19%, and thus validated the IDMS method for paraquat and diquat analysis. As expected, environmental water samples prepared using this method achieved much better sensitivity (MDL=0.1 μg/l diquat and 0.2 μg/l paraquat) than can be achieved using the IP UV method [6] which has typical MDLs of 0.5 μg/l for diquat and 1 μg/l for paraquat. The use of resin SPE cartridges with the LC–ESI-IDMS analysis of paraquat and diquat allowed a significant improvement in data quality and operation efficiency.

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